Seeking the Sensitivity Limit

Eric O. Potma

UC Irvine

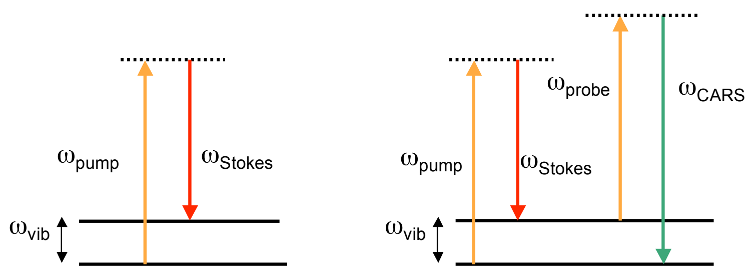
Sensitivity in CARS

- sensitivity in regular CARS microscopy
- signal levels & photo damage
- the nonresonant background
- background suppression
- advanced techniques

Why CARS again?

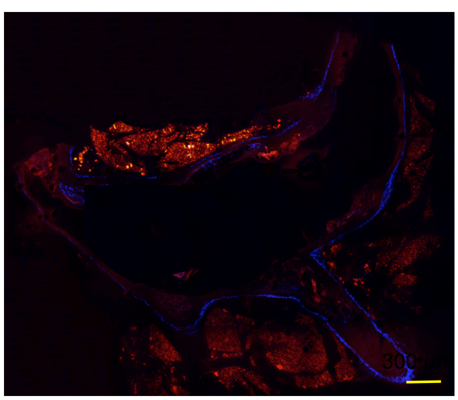
Raman

CARS

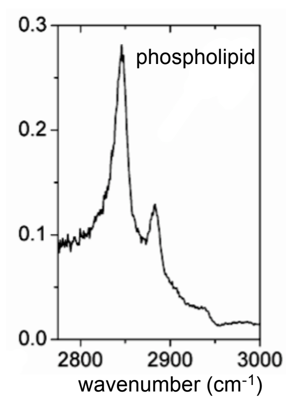


Two different modes

Microscopy



Microspectroscopy



spatial information

spectrally 'narrow' pulses

very short pixel dwell times

spectral information

spectrally broad pulses

longer pixel dwell times

Current status of 'regular' CARS

Spectral bands: CH, CD, OH, CN, C=C aromatic ring, Amide I etc.

Spatial resolution: 0.25 μm lateral, 0.7 μm axial

(0.05 μm lateral in near-field)

Scanning speed: 30 frames/sec (E-CARS)

Powers at sample: 1-50 mW for ~5 ps pulses

Det. Sensitivity: ~10^5 CH_2 modes in focus

Limited by: nonresonant background, signal strength

Some numbers

Regular CARS detection sensitivity

Lipid bilayer:

E-CARS:

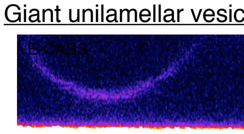
4.4 x 10^6 CH_2 modes in focus

~8 x 10^3 CARS photons/second (25 mW pump @ 76 MHz)

1 x 10^{-4} photons per shot (detected)

1 x 10^{-13} photon conversion efficiency

Giant unilamellar vesicle



F-CARS:

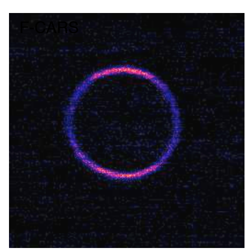
10 x 10^6 CH_2 modes in focus

~1 x 10^7 CARS photons/second (25 mW pump @ 76 MHz)

1.3 x 10^{-1} photons per shot (detected)

1.3 x 10^{-10} photon conversion efficiency

Lysed red blood cell



We want more photons!

CARS signal levels

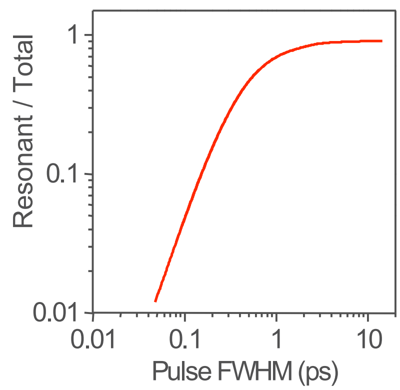
Total collected CARS signal is function of:

dwell time t_{scan}, *pulse repetition rate* f, *pulse width* τ_{pulse} *and time averaged excitation power* $\langle P(t) \rangle$

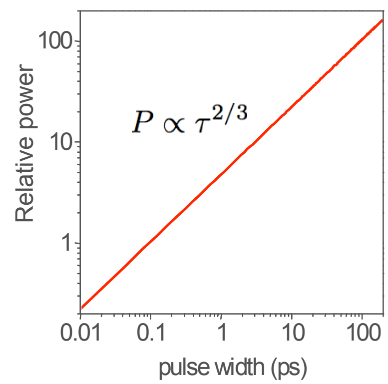
$$S_{CARS} \propto \frac{t_{scan}}{(f\tau_{pulse})^2} \langle P(t) \rangle^3$$

Pulse width

Signal to background



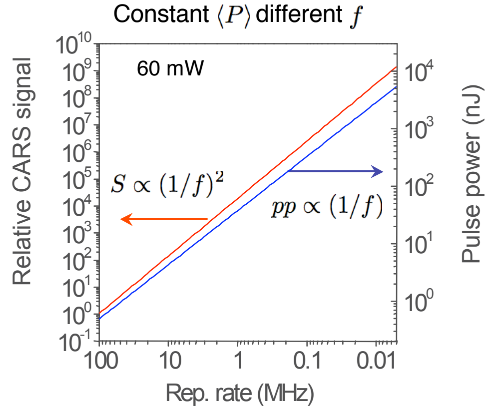
Excitation power relative
to signal at 100 fs



Repetition Rate

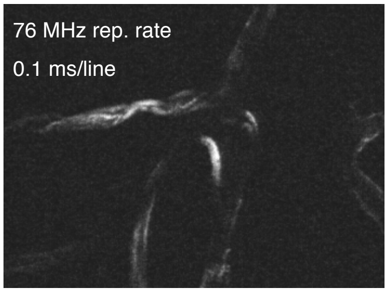
$$S_{CARS} \propto \frac{t_{scan}}{(f\tau_{pulse})^2}\langle P(t)\rangle^3$$

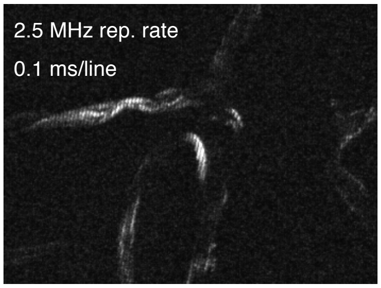
Constant $\langle P \rangle$ different f



Scanning speed vs rep. rate

A minimum of 1 shot per pixel is required to avoid pattern formation.





Hitting the roof: Photo damage

$$S_{CARS} \propto \frac{t_{scan}}{(f\tau_{pulse})^2} \langle P(t) \rangle^3$$

<u>Two main mechanisms for photo damage:</u>

- linear absorption: linear heating

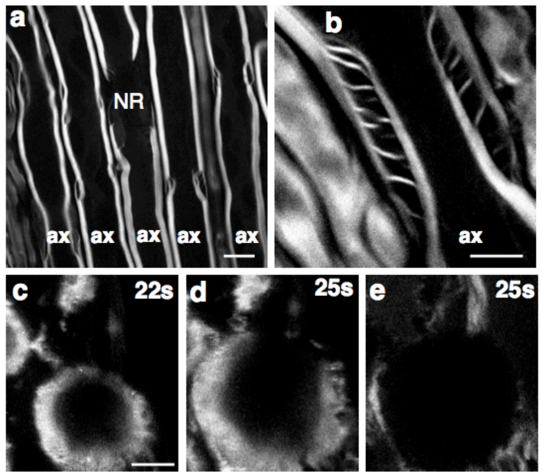
100 mW of 850 nm through 1.2W NA lens gives rise to heating of 0.2 K in aqueous medium (Schole & Hell, Opt. Lett. 23, 325 (1998))

- nonlinear absorption: heating and photo-chemically induced changes

Multiple (nonlinear) mechanisms, molecule and sample specific

Types of Photo damage

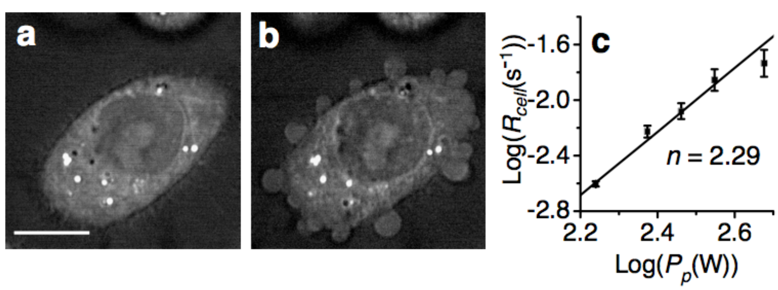
Plasma formation and Raman heating



Fu et al., Opt. Express 14, 3942 (2006)

Types of Photo damage

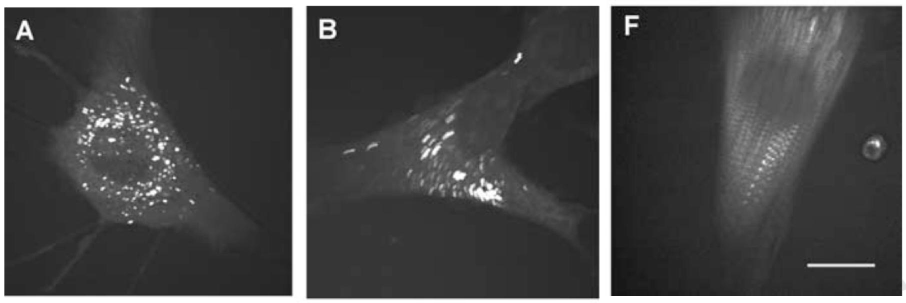
Cellular membrane blebbing



Fu et al., Opt. Express 14, 3942 (2006)

Types of Photo damage

Cellular retraction



2 nJ, 1 MHz | 3 nJ, 1 MHz | 0.15 nJ, 80 MHz

Nan et al., Biophys J. 91(2), (2006)

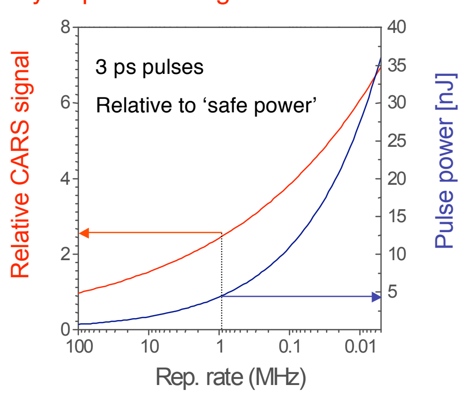
Safe limits

According to Hopt & Neher (*Biophys. J. 80, 2029 (2002)*)

$$\#\ scans \propto \frac{(f\tau_{pulse})^{1.5}}{t_{scan}\,\langle P(t)\rangle^{2.5}}$$

'Safe Power': ~10 mW, 80 fs, 80 MHz

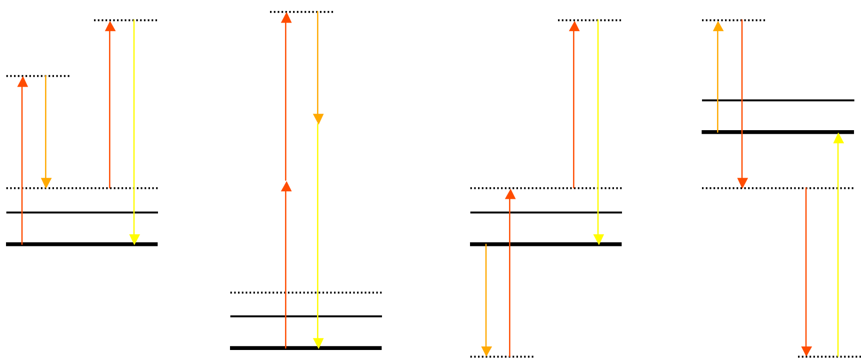
if nonlinearity of photodamage < CARS nonlinearity (n=3)



Nonresonant background

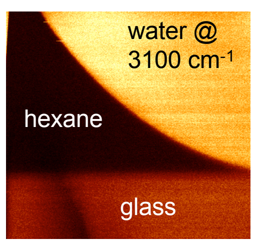
What is it?

Oscillating electron clouds also radiate at CARS frequency

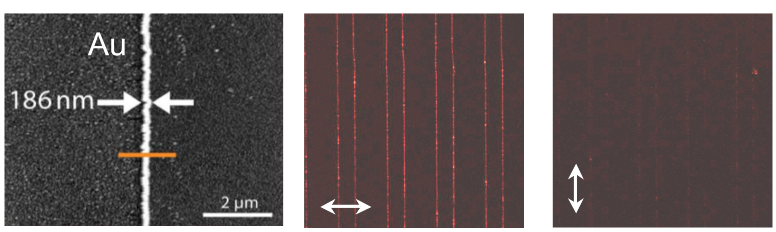


$\chi^{(3)}(\omega_{CARS})$ has 24 terms: 4 resonant and 20 nonresonant
(only 1/6 is resonant)

Different $\chi_{nr}^{(3)}$ signals



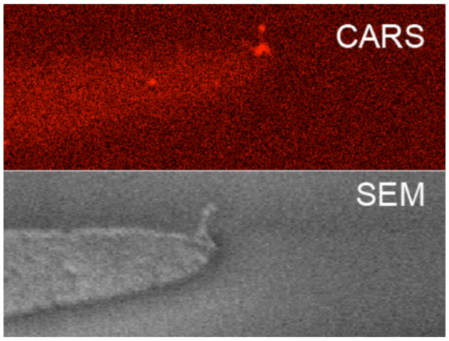
nanometal @ 2845 cm^{-1}



No molecular vibrations!

$\chi_{nr}^{(3)}$ can be strong!

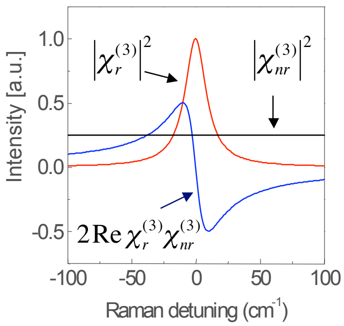
coherent anti-Stokes scattering from single nanotubes



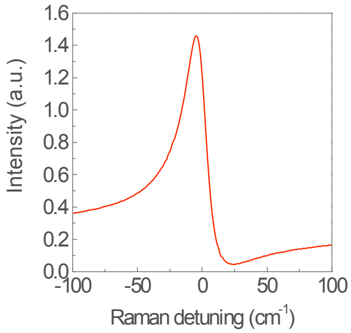
Not just an addition

$$S_{CARS} \propto \left|\chi_{nr}^{(3)}\right|^2 + \left|\chi_{r}^{(3)}\right|^2 + 2\chi_{nr}^{(3)} Re\left\{\chi_{r}^{(3)}\right\}$$

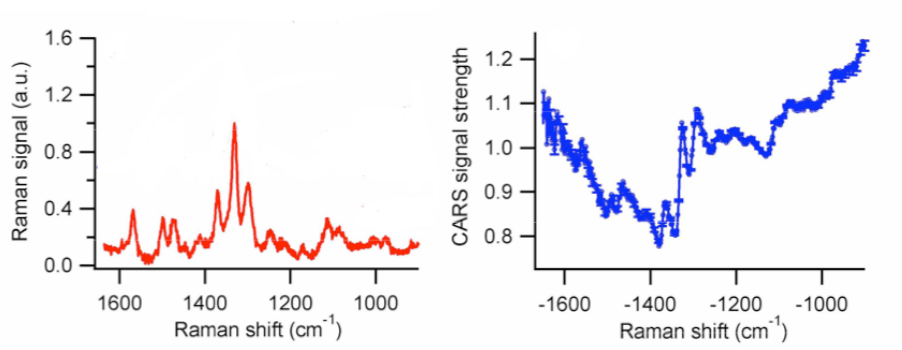
components



total CARS signal



Disappearance of bands

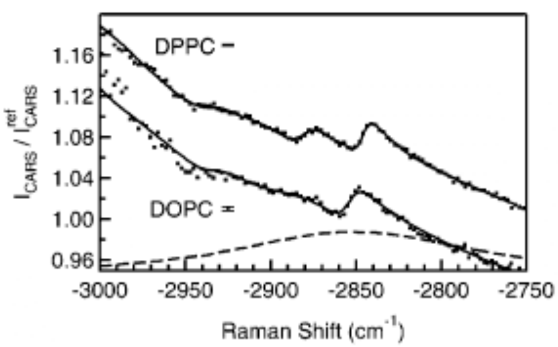


Aqueous ATP/ADP/AMP mixture

Vartiainen et al, Opt. Express 14, 3622 (2006)

Always a problem?

$$S_{CARS} \propto \left|\chi_{nr}^{(3)}\right|^2 + \left|\chi_r^{(3)}\right|^2 + 2\chi_{nr}^{(3)} Re\left\{\chi_r^{(3)}\right\}$$



Wurpel et al., J. Phys. Chem. B 108, 3400 (2004)

Getting rid of it

Partially:

- subtraction of off-resonant component
- Epi-CARS for suppressing solvent contribution
- spectral phase and modulation techniques

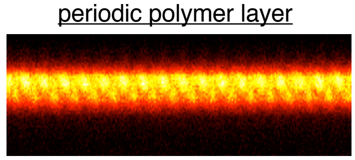
Entirely:

- Polarization sensitive CARS
- Time-resolved CARS
- Phase sensitive detection

Poor man's solution

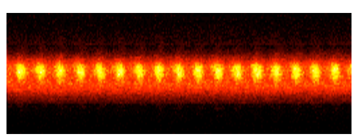
periodic polymer layer

on-resonance



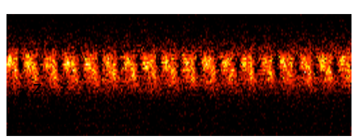
$$|\chi_{nr}^{(3)}|^2 + |\chi_r^{(3)}|^2 + 2\mathrm{Re}\,\chi_r^{(3)}\chi_{nr}^{(3)}$$

off-resonance



$$|\chi_{nr}^{(3)}|^2$$

on-resonance - off-resonance

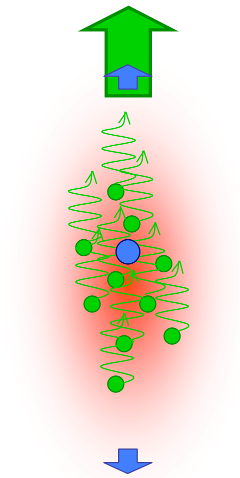


$$|\chi_r^{(3)}|^2 + 2\mathrm{Re}\,\chi_r^{(3)}\chi_{nr}^{(3)}$$

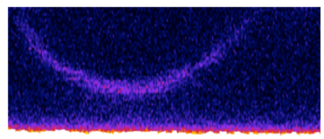
Tert-butoxyl skeletal mode @ 891 cm^{-1}

What E-CARS does

E-CARS detects $\chi^{(3)}$ discontinuities



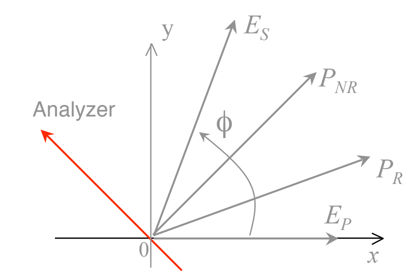
$$\left|\chi^{(3)}_{nr,solvent}\right|^2 + \left|\chi^{(3)}_{nr,object}\right|^2 + \left|\chi^{(3)}_{r}\right|^2 + 2\mathrm{Re}\,\chi^{(3)}_{r}\chi^{(3)}_{nr(solvent+object)}$$



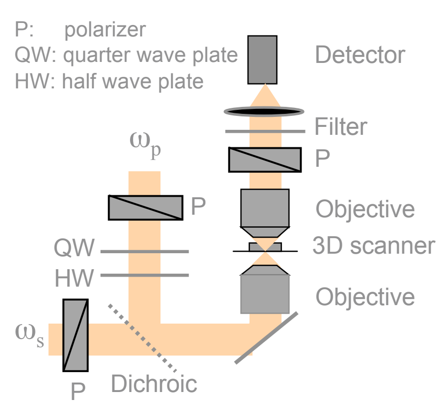
$$\left|\chi^{(3)}_{nr,object}\right|^2 + \left|\chi^{(3)}_{r}\right|^2 + 2\mathrm{Re}\,\chi^{(3)}_{r}\chi^{(3)}_{nr,object}$$

- Solvent background is completely rejected
- For objects > $\lambda_{CARS}/4$, destructive interference reduces the signal in E-CARS

Polarization CARS

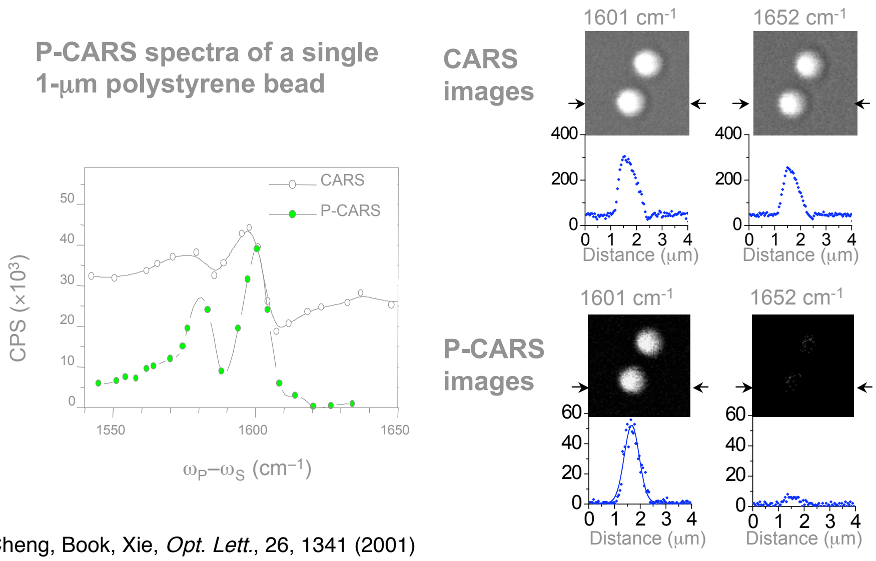


The optimal value for ϕ is 71.6 °



Cheng, Book, Xie, *Opt. Lett.*, 26, 1341 (2001)

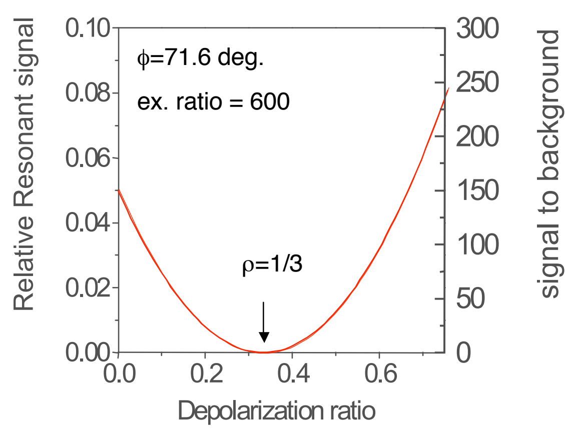
P-CARS



Cheng, Book, Xie, *Opt. Lett.*, 26, 1341 (2001)

P-CARS

- P-CARS rejects all the nonresonant background
- Improves signal/background, decreases absolute signal strength
- Limited by inherent depolarization and birefringence

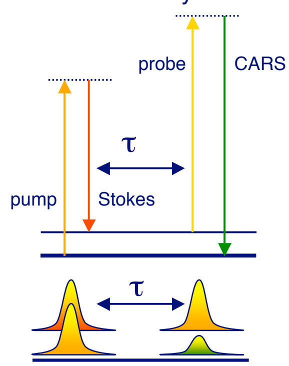


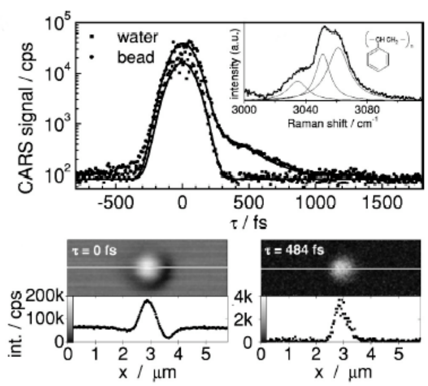
CH_2 stretch @ 2845 cm^{-1}:

ρ = 0.21

Time-resolved CARS

Probing the Raman free induction decay





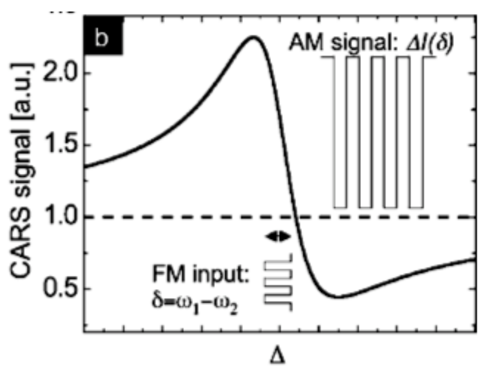
- complex 3-color configuration
- signal at longer time delays is small

Volkmer et al., Appl. Phys. Lett. 80, 1505 (2002)

Advanced methods

- Frequency modulated CARS
- Broadband CARS with phase shaping
- narrow band CARS interferometry

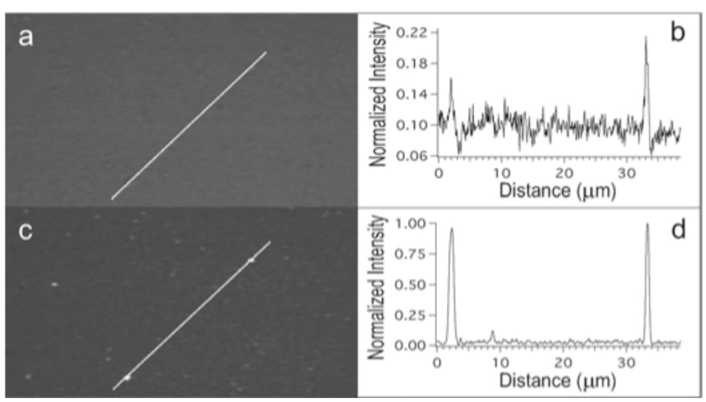
FM-CARS



Sensitive to: $\left|\chi_r^{(3)}\right|^2 + 2\mathrm{Re}\,\chi_r^{(3)}\chi_{nr}^{(3)}$

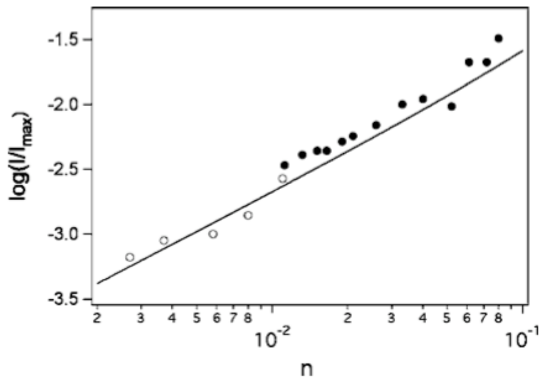
At low concentration: $2\mathrm{Re}\,\chi_r^{(3)}\chi_{nr}^{(3)}$

FM-CARS



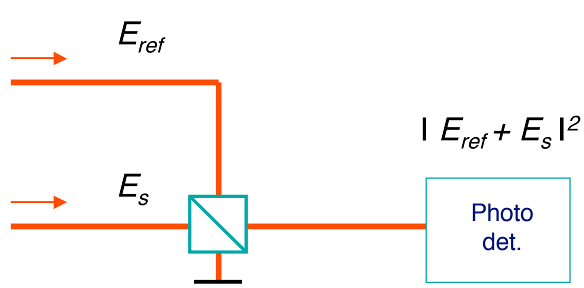
Ganikhanov et al., Opt. Lett. 31, 1872-1874 (2006).

FM-CARS



Using phase:

CARS interferometry



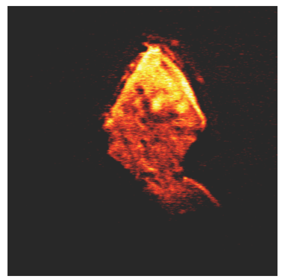
$$|E_s|^2 + |E_{ref}|^2 + 2E_sE_{ref}\cos\phi$$

Narrow band interferometry

$$S \propto I_{ref} + I_s + 2E_{ref}E_s\left[\chi^{(3)}_{nr} + \mathrm{Re}\left\{\chi^{(3)}_r\right\}\right]\cos\Phi + \boxed{2E_{ref}E_s\,\mathrm{Im}\left\{\chi^{(3)}_r\right\}\sin\Phi}$$

Regular CARS

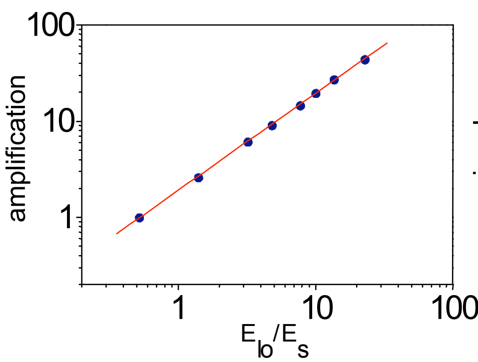
Interferometric CARS

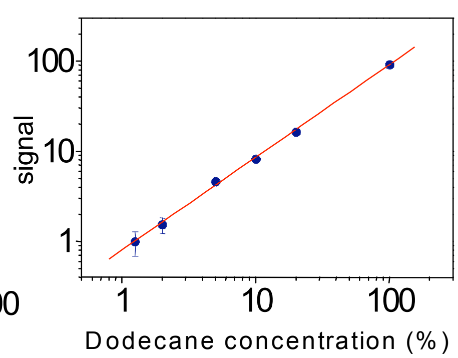


Casein protein aggregate (CH_3 @ 2940 cm^{-1})

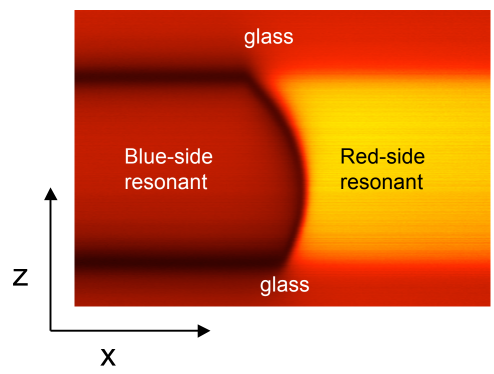
What interferometry offers

- Linear in concentration
- Linear in Im $\{\chi^{(3)}\}$, proportional to spontaneous Raman
- Linear amplification through varying E_{ref}

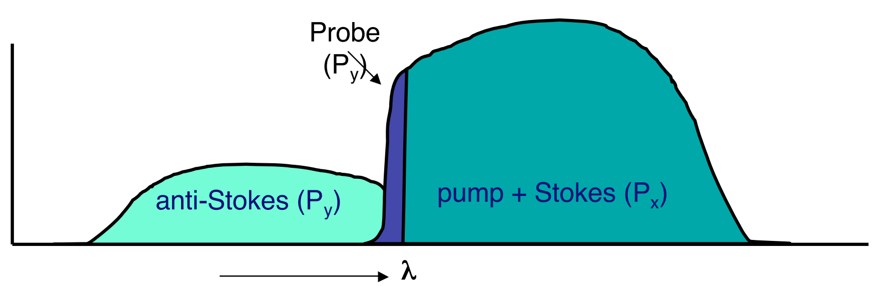




inherent interferometric effect



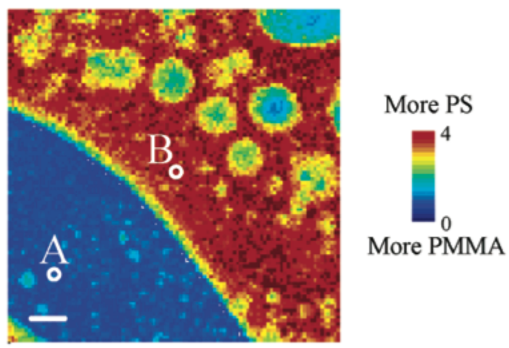
Broadband interferometry

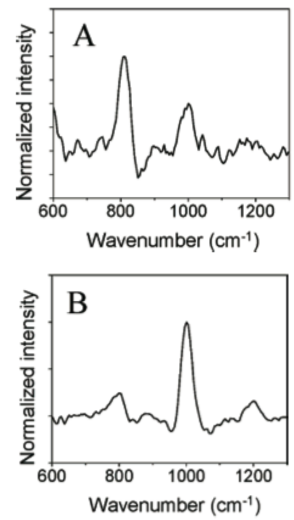


- High spectral resolution
- background suppression through phase shaping

Oron et al., Phys. Rev. Lett. 90, 213902 (2003)

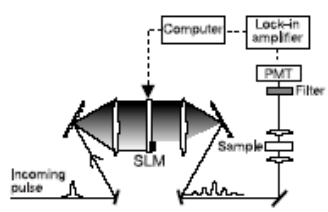
Broadband interferometric imaging

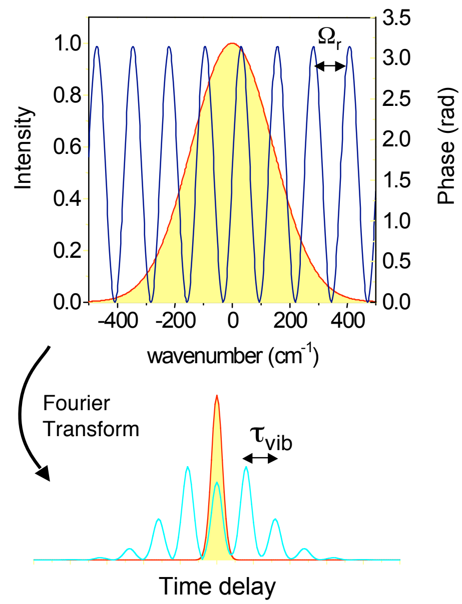




Lim et al.,JPC B 110, 5196 (2006)

Broadband phase shaping



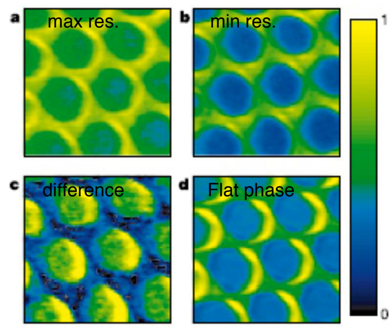


Dudovich et al., Nature 418, 512 (2002)

Background suppression

with spectral phase shaping

Glass capillaries filled with CH_2Br_2

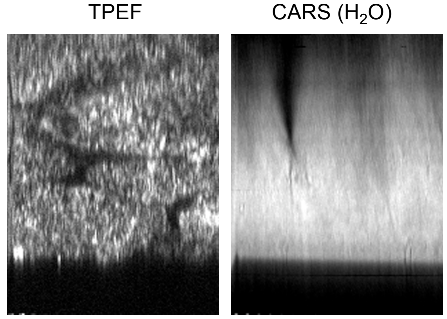


- spectral resolution limited by SLM
- nonresonant background at Ω_r cannot be suppressed

$\chi^{(3)}$ and $\chi^{(1)}$

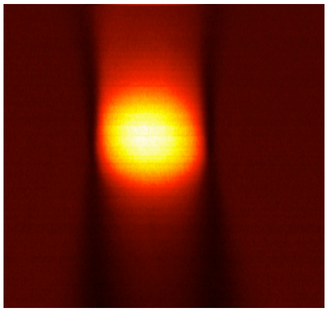
TPEF

CARS (H_2O)

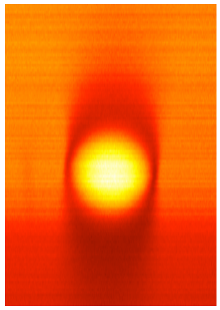


$\chi^{(3)}$ and $\chi^{(1)}$

dodecane droplet in H_2O



paraffin droplet in d-DMSO



Faster acquisition

- Point-illumination
  - resonant Galvos
  - spinning polygon
  - multifocal: scanning array
  - multifocal: microlens spinning disk

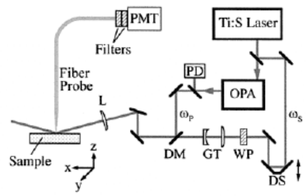
- Wide-field illumination

Retaining image brightness with faster acquisition times always requires higher illumination powers at the sample

Higher resolution

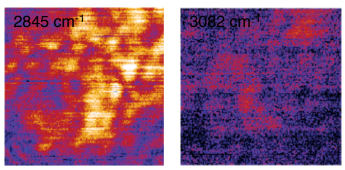
near field methods

Fiber probe detected



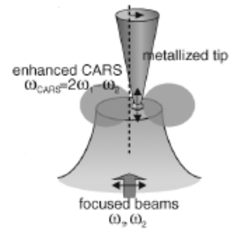
Resolution ~ 130 nm

Human hepatocyte (4.3 x 4.3 μm)



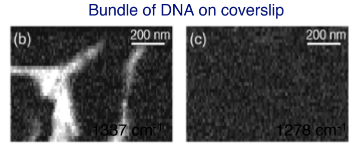
Schaller et al., J. Phys. Chem. B 106, 8489 (2002)

Tip enhanced NSOM



Resolution < 50 nm

Bundle of DNA on coverslip



Ichimura et al., Phys. Rev. Lett. 92, 220801 (2004)

Suitable for thin (< 1 μm) samples on surfaces

Recap

Brightest signals, fastest acquisition:

High rep rate (1-100 MHz) ps light source (< 50 mW)

Point-scanning F-CARS mode: > 1 photon/shot

> 30 frames/s

Economic rejection of solvent background:

E-CARS (signal reduction 1-10^3 relative to F-CARS)

Highest sensitivity:

FM-CARS for isolated vibrational bands

Highest spatial resolution:

Tip-enhanced near field CARS microscopy with resolution < 50 nm

Thanks!